SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES, LEGENDS AND TABLE

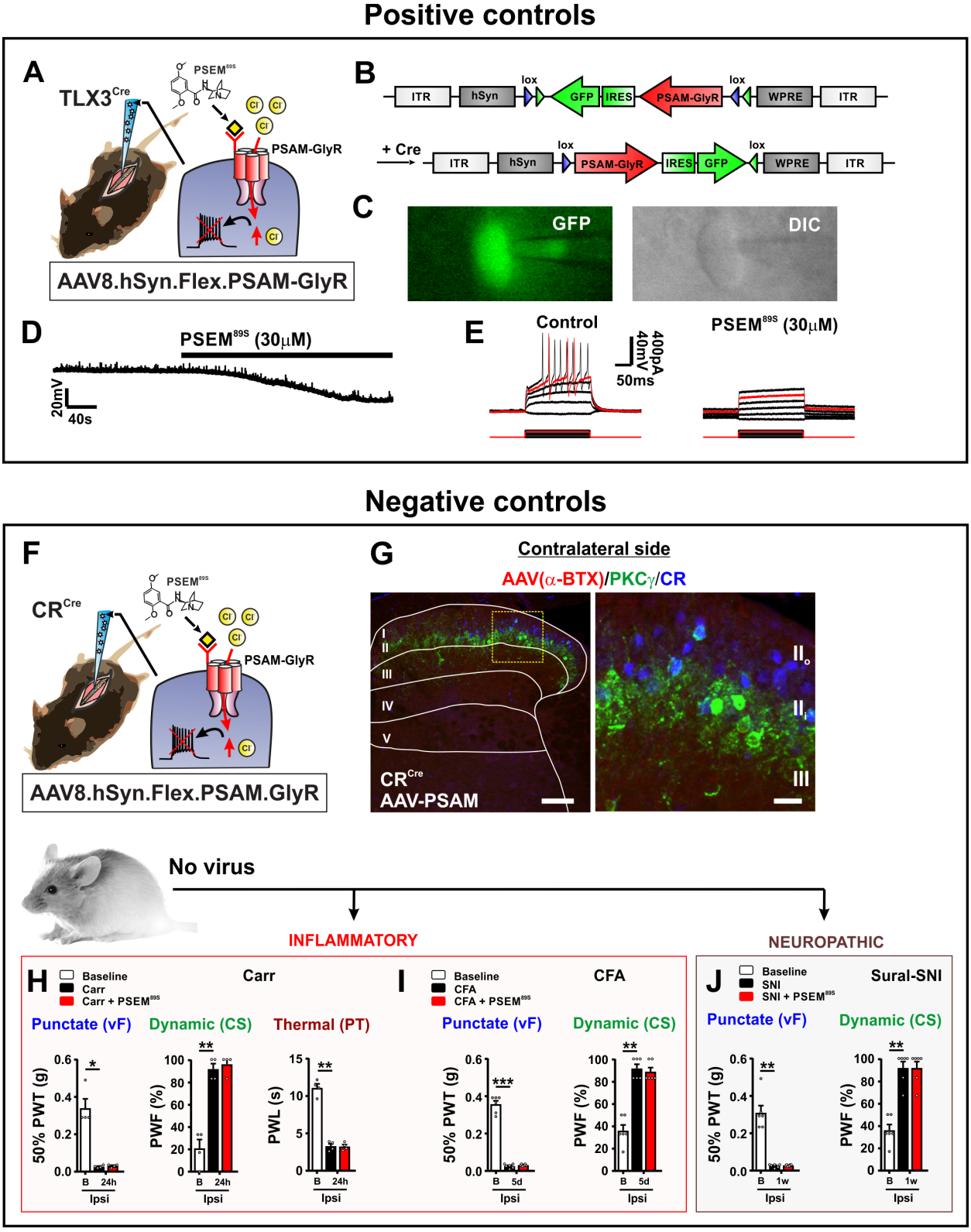

Figure S1. Refers to Figure 2. Positive and negative controls for PSAM-GlyR and PSEM^{89S}.

(A-B) Schematic of intraspinal injection of AAV8.hSyn.FLEX.PSAM-GlyR into $Tlx3^{Cre}$ mice for patch clamp recordings in lumbar spinal cord slices. Diagram of the hSyn.FLEX.PSAM-GlyR-IRES-GFP viral construct. GFP is produced from the same transcript.

(C-D) Electrode impaling a GFP^+ cell (also shown as DIC image) in a transverse slice of the dorsal horn. Bath application of 30 μM $PSEM^{89S}$ induces a sustained hyperpolarization specifically in GFP-expressing cells.

(E) Current injected into GFP^+ cell produces action potentials that are completely blocked after application of $PSEM^{89S}$ (30 μM) to the slice.

(F-G) Control for the specificity of α-BTX-Alexa647 staining. Mouse unilaterally injected with AAV8.hSyn.FLEX.PSAM-GlyR virus. Contralateral dorsal horn lacks staining for α-BTX-Alexa647(red). Calretinin (blue); PKCγ (green). Yellow box indicates area of inset. Scale bars = 100 μm and 20 μm.

(H-J) injection of $PSEM^{89S}$ into control mice lacking the receptor has no effect on punctate or dynamic allodynia induced by carrageenan, CFA or SNI. It also has no effect on heat hypersensitivity in the carrageenan model. N=4-6 mice per condition.

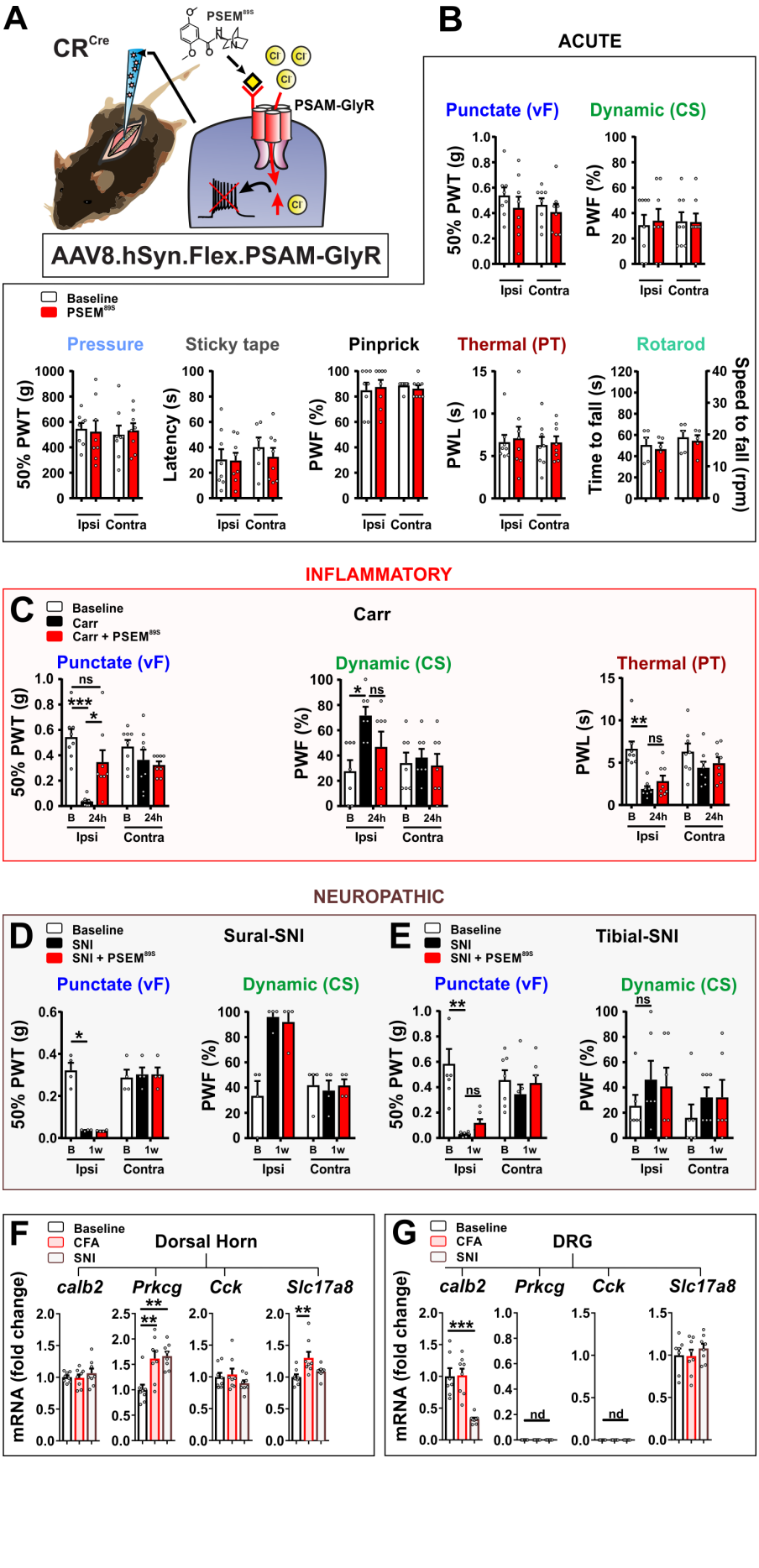
A
CR^Cre
PSEM^89S
PSAM-GlyR
AAV8.hSyn.Flex.PSAM-GlyR
B
ACUTE
Punctate (vF)
Dynamic (CS)
50% PWT (g)
PWF (%)
Ipsi Contra
Baseline
PSEM^89S
Pressure
Sticky tape
Pinprick
Thermal (PT)
Rotarod
Latency (s)
PWL (s)
Time to fall (s)
Speed to fall (rpm)
INFLAMMATORY
C
Baseline
Carr
Carr + PSEM^89S
Punctate (vF)
Dynamic (CS)
Thermal (PT)
B 24h
Ipsi Contra
NEUROPATHIC
D
Baseline
SNI
SNI + PSEM^89S
Sural-SNI
Punctate (vF)
Dynamic (CS)
B 1w
E
Tibial-SNI
F
Baseline
CFA
SNI
Dorsal Horn
calb2
Prkcg
Cck
Slc17a8
mRNA (fold change)
G
DRG
nd

Figure S2. Refers to Figure 2. Role of virally targeted lamina II calretinin neurons in acute and persistent pain behaviors.

(A) Schematic of intraspinal injection of AAV8.hSyn.FLEX.PSAM-GlyR virus into the dorsal horn of CR^{Cre} mice.

(B) Injection of $PSEM^{89S}$ (red bars) has no effect on acute von Frey (vF) threshold, response to cotton swab (CS), pressure with calibrated calipers, latency to remove sticky tape or pinprick response (white bars). Hargreaves latencies in the plantar test (PT) are also unchanged. Motor behavior measured by rotarod is also not altered by inhibition of the targeted CR neurons. N=8 mice per group.

(C) In the carrageenan model of inflammatory pain, injection of $PSEM^{89S}$ reverses punctate ($p=0.0407$), but not dynamic allodynia or heat hypersensitivity. N= 8 mice per group. Measurements before injury are white, after injury are black and after injury + $PSEM^{89S}$ are red.

(D-E) In the SNI models of neuropathic pain, injection of $PSEM^{89S}$ has no effect on punctate or dynamic allodynia in the sural model and no effect on punctate allodynia in the tibial model, both measured at 1 week after the injury. The tibial model does not produce dynamic allodynia. N= 4-7 mice per group. Measurements before injury are white, after injury are black and after injury + $PSEM^{89S}$ are red.

(F) RT-PCR of the dorsal horn shows that compared to saline injected mice (Black bars), the levels of *Prkcg* mRNA (PKCγ) are increased (~1.5 fold) 5 days after CFA (red bars) and 1 week after sural-SNI (brown bars). A modest increase in *Slc17A8* mRNA (VGLUT3) is detected after CFA. No changes are detected for *Cck* (CCK) or *Calb2* (CR) mRNAs. N= 8 mice per condition.

(G) RT-PCR of DRG shows a significant decrease in *Calb2* mRNA (CR) 1 week after SNI. No changes are detected for *Slc17A8* (VGLUT3). Neither *Prkcg* (PKCγ) *nor Cck* (CCK) mRNAs are detected in DRG in any of these conditions. N= 8 mice per condition.

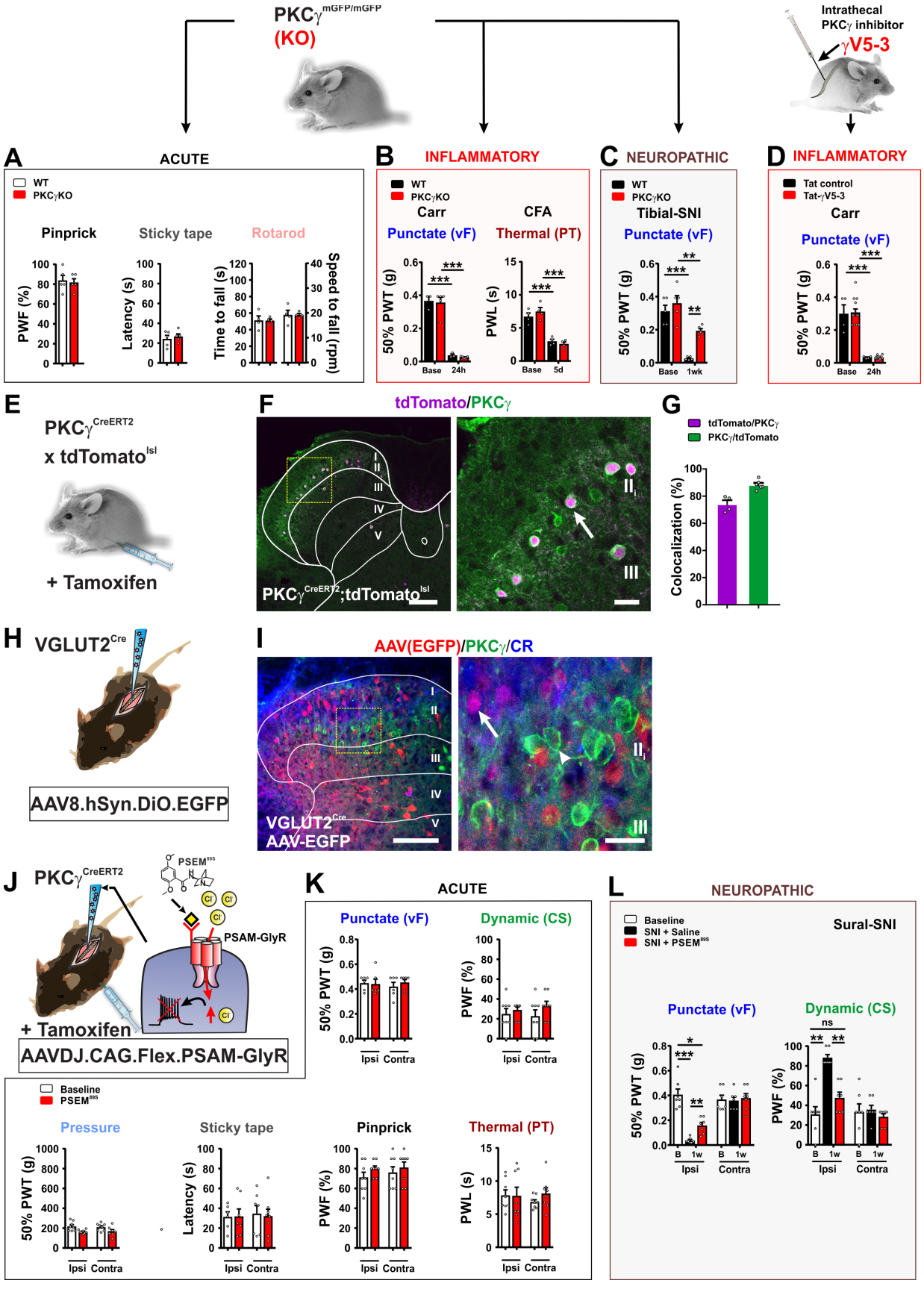
PKCγ mGFP/mGFP (KO)
Intrathecal PKCγ inhibitor
γV5-3
A ACUTE
WT
PKCγKO
Pinprick
Sticky tape
Rotarod
PWF (%)
Latency (s)
Time to fall (s)
Speed to fall (rpm)
B INFLAMMATORY
Carr
CFA
Punctate (vF)
Thermal (PT)
50% PWT (g)
PWL (s)
Base
24h
5d
C NEUROPATHIC
Tibial-SNI
1wk
D INFLAMMATORY
Tat control
Tat-γV5-3
E
PKCγ CreERT2
x tdTomato lsl
+ Tamoxifen
F tdTomato/PKCγ
PKCγ CreERT2;tdTomato lsl
G
tdTomato/PKCγ
PKCγ/tdTomato
Colocalization (%)
H VGLUT2 Cre
AAV8.hSyn.DiO.EGFP
I AAV(EGFP)/PKCγ/CR
VGLUT2 Cre
AAV-EGFP
J PKCγ CreERT2
PSEM 89S
PSAM-GlyR
+ Tamoxifen
AAVDJ.CAG.Flex.PSAM-GlyR
K ACUTE
Punctate (vF)
Dynamic (CS)
Ipsi
Contra
Baseline
PSEM 89S
Pressure
Sticky tape
Pinprick
Thermal (PT)
L NEUROPATHIC
Baseline
SNI + Saline
SNI + PSEM 89S
Sural-SNI
Punctate (vF)
Dynamic (CS)
ns

Figure S3. Refers to Figure 4. Role of PKCγ kinase and neurons in acute and persistent pain behaviors.

(A) Response to pinprick and latency to remove a piece of sticky tape are similar between PKCγ KO mice (red bars) and wildtype littermates (white bars). Rotarod performance is also similar between the two genotypes. N= 4 mice per genotype.

(B) In the CFA model of inflammatory pain, punctate allodynia and heat hyperalgesia do not differ between PKCγ KO (red bars) and WT littermates (black bars). N=4 mice per genotype.

(C) In the tibial-SNI model, punctate mechanical allodynia is significantly reduced in PKCγ KOs (red bars) compared to WT littermates (black bars) ($p=0.0024$). N=5 mice per genotype.

(D) Injection of the PKCγ inhibitor tat-γV5-3 (100 pmoles, *i.t.)* (red bars) has no effect on punctate allodynia induced by the carrageenan model of inflammatory pain. Tat peptide (100 pmoles, *i.t.*) (black bars) served as control. N=14 mice for tat-γV5-3, N=4 mice for tat-control peptide.

(E-G) $PKC\gamma^{CreERT2}$ Ai14-tdTomato reporter mice show expression of PKCγ in ~90% of $tdTomato^{+}$ neurons (magenta) and expression of tdTomato in ~75% of $PKC\gamma^{+}$ neurons. n= 4 slices, N=2 mice. Yellow box shows location of insert. Arrow shows example of colocalized cell. Scale bars = 100 μm and 20 μm.

(H) Schematic of intraspinal injection of $VGLUT2^{Cre}$ mouse with AAV8.hSyn.DIO.EGFP virus.

(I) Spinal cord neurons expressing EGFP (red) overlap with CR (blue, arrow) but not PKCγ (green, arrowhead) population. Scale bars = 50 μm and 10 μm.

(J) Schematic of intraspinal injection of PKC$\gamma^{CreERT2}$ mouse with AAVDJ.CAG.FLEX.PSAM-GlyR virus. Injection of tamoxifen is required for Cre recombination to allow expression of PSAM-GlyR.

(K) Injection of PSEM^{89S} (red bars) has no effect on acute somatosensory behaviors (white bars) including von Frey (vF) threshold, cotton swab (CS) responsiveness, paw pressure threshold, latency to remove sticky tape, pinprick responsiveness or heat sensitivity measured with the plantar test of Hargreaves assay (PT). N= 7 mice.

(L) One week after induction of the sural-SNI model of neuropathic pain, injection of PSEM^{89S} partially reverses punctate (p=0.0038) and completely reverses dynamic (p=0018) allodynia. N= 7 mice per group. Measurements before injury saline are white, after injury + saline are black and after injury + PSEM^{89S} are red.

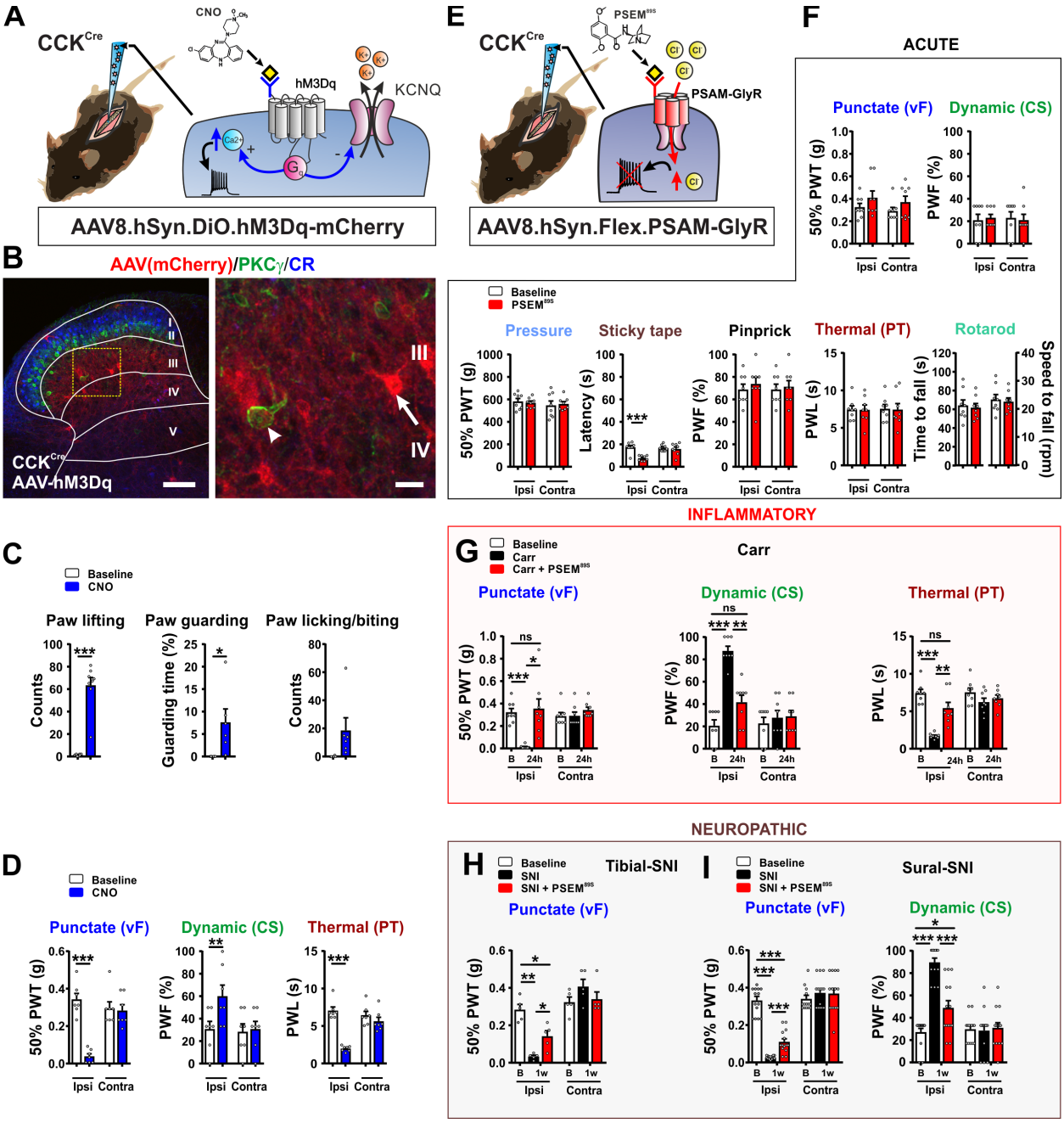

Figure S4. Refers to Figure 6. The role of targeted CCK neurons in acute and persistent pain behaviors.

(A) Schematic of injection of AAV8.hSyn.DIO.hM3Dq-mCherry virus into the dorsal horn of CCK^{Cre} mice. CNO depolarizes neurons expressing hM3Dq.

(B) Dorsal horn of an AAV8.hSyn.DIO.hM3Dq-mCherry injected CCK^{Cre} mouse shows expression of mCherry (red) mainly in laminae III-V. No co-localization was observed with CR (blue) or PKCγ (green). Scale bars = 50 μm and 10 μm.

(C) Spontaneous behaviors such as paw shaking, guarding and licking (white bars) are increased only at the ipsilateral hind paw after injection of CNO (5 mg/kg, *i.p.)* (blue bars).

(D) Injection of CNO (blue bars) causes a significant decrease in the von Frey threshold, increase in response to a cotton swab and decrease in paw withdrawal latency in the plantar test of Hargreaves assay compared to baseline (white bars) only at the ipsilateral hind paw.

(E) Schematic of intraspinal injection of AAV8.hSyn.FLEX.PSAM-GlyR into CCK^{Cre} mice

(F) Injection of $PSEM^{89S}$ (red bars) has no effect on acute von Frey threshold, response to cotton swab, pressure with calibrated calipers or pinprick, but does increase response to sticky tape (p=0.0006) compared to before ligand (white bars). Hargreaves latencies are unchanged. Performance on the rotarod is also not altered by injection of $PSEM^{89S}$. N= 8 mice for each test.

(G) Injection of $PSEM^{89S}$ (red bars) completely reverses punctate (p=0.0159) and dynamic (p=0.0037) allodynia as well as heat hypersensitivity (p=0.0045) induced by the carrageenan model of inflammatory pain. N=8 mice.

(H) Injection of $PSEM^{89S}$ at 1 week after tibial-SNI partially reverses punctate allodynia (p=0.0283). N= 5 mice

(I) $PSEM^{89S}$ also partially reverses punctate (p=0.0003) and dynamic (p=0.0001) allodynia 1 week after sural-SNI. N=14 mice

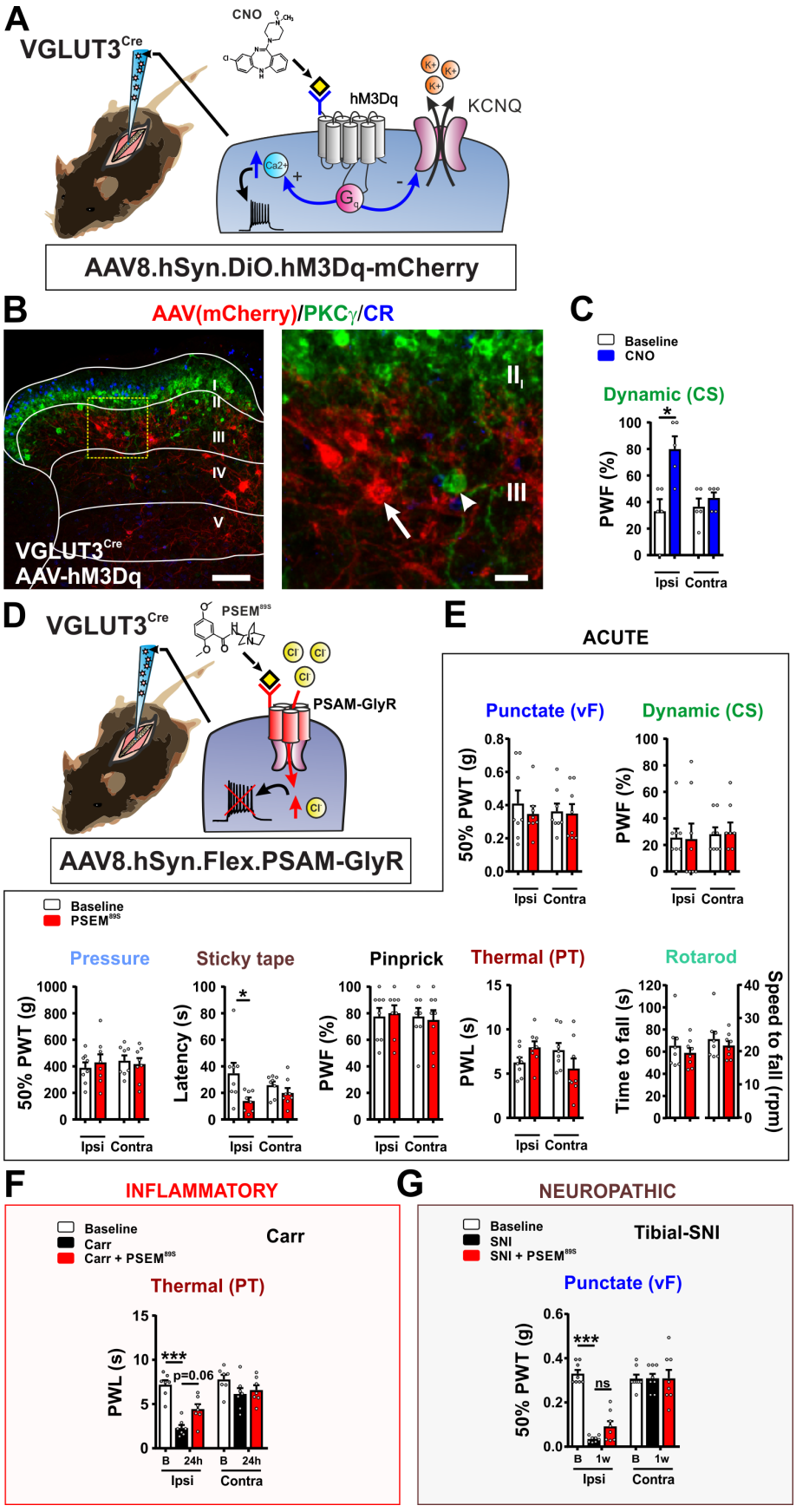
A
VGLUT3Cre
CNO
hM3Dq
KCNQ
Ca2+
Gq
AAV8.hSyn.DiO.hM3Dq-mCherry
B
AAV(mCherry)/PKCγ/CR
I
II
III
IV
V
VGLUT3Cre
AAV-hM3Dq
IIi
III
C
Baseline
CNO
Dynamic (CS)
PWF (%)
Ipsi Contra
D
VGLUT3Cre
PSEM89S
PSAM-GlyR
AAV8.hSyn.Flex.PSAM-GlyR
E
ACUTE
Punctate (vF)
Dynamic (CS)
50% PWT (g)
PWF (%)
Baseline
PSEM89S
Pressure
Sticky tape
Pinprick
Thermal (PT)
Rotarod
50% PWT (g)
Latency (s)
PWF (%)
PWL (s)
Time to fall (s)
Speed to fall (rpm)
F
INFLAMMATORY
Baseline
Carr
Carr + PSEM89S
Carr
Thermal (PT)
PWL (s)
***
p=0.06
B 24h B 24h
Ipsi Contra
G
NEUROPATHIC
Baseline
SNI
SNI + PSEM89S
Tibial-SNI
Punctate (vF)
50% PWT (g)
***
ns
B 1w B 1w
Ipsi Contra

Figure S5. Refers to Figure 7. The role of targeted VGLUT3 neurons in acute and persistent pain behaviors.

(A-B) Schematic of intraspinal injection of AAV8.hSyn.DIO.hM3Dq-mCherry into VGLUT3^{Cre} mice. Dorsal horn shows expression of mCherry^{+} neurons (red) concentrated in laminae III-IV with sparse cells in laminae II and V. No colocalization was observed with calretinin (blue) or PKCγ (green). Scale bar = 50 μm and 10 μm.

(C) Response of the ipsilateral hind paw to a cotton swab is significantly increased after injection of CNO (5 mg/kg, *i.p.*) (blue bars) compared to before CNO (white bars) (p=0.0252). No change on the contralateral side. N= 5 mice.

(D) Schematic of intraspinal injection of AAV8.hSyn.FLEX.PSAM-GlyR virus into VGLUT3^{Cre} mice.

(E) Injection of PSEM^{89S} (red bars) does not alter acute von Frey threshold, response to cotton swab, pressure with calibrated calipers or pinprick, but does shorten the latency to respond to a piece of sticky tape (p=0.0251) compared to without ligand (white bars). Hargreaves latencies are also unchanged. Performance on the rotarod is also not altered in these mice after PSEM^{89S} injection. N=8 mice in each test.

(F) PSEM^{89S} injection does not reduce heat hypersensitivity induced by the carrageenan model of inflammatory pain (p=0.0625). N=7 mice.

(G) Injection of PSEM^{89S} has no effect on punctate allodynia in the tibial-SNI model at 1 week. N=8 mice.

Table S1. Refers to STAR Methods. Primers used for qPCR.

qPCR primers	Forward (5'-3')	Reverse (5'-3')
Cck	TAGCGCGATACATCCAGCAG	AAATCCATCCAGCCCATGTAG
Calb2	GGCATGATGTCCAAGAGCGA	TTCCGCCAAGCCTCCATAAA
Slc17a8	GTGACACAAATTCCCGGTGG	TACTGCACCAATACCCCTGC
Prkcg	TACAAGTTACTGAACCAGGAGG	GCTCTGCCAGCATTACCTTC
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il6	TTCCTACCCCAATTTCCAAT	CCTTCTGTGACTCCAGCTTATC
Tnf	AGGGATGAGAAGTTCCCAAATG	GGCTTGTCACTCGAATTTTGAGA
Ly6g	AACACAACTACCTGCCCCTTC	CACGTTGACAGCATTACCAGTG
Gapdh	GGGTGTGAACCACGAGAAAT	CCTTCCACAATGCCAAAGTT